CTH-03

IN THE UNITED STATES DESIGNATED OFFICE

Applicants	Neil A. Williams, Timothy R. Hirst, and John Bienenstock
Serial No.	Filing Date: 10 July 2000
Title of Application	**Agent for Treating Allergic or Hypersensitivity Condition**

Commissioner of Patents
and Trademarks
Washington, DC 20231

BOX PCT, ATTENTION DO/US

Transmittal Letter to the U.S. Designated Office (DO/US)
Entry into the U.S. National Stage Under Chapter II

Applicant herewith submits to the United States Designated Office (DO/US) the below listed items under 35 U.S.C. 371. The below-checked items are being transmitted by 30 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date, as evidenced by the documents cited below and enclosed herewith.

This is an express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

1. A copy of the International Application as filed (35 U.S.C. 371(c)(2)):

[xx]	is transmitted herewith.
[ ]	is not required as the application was filed with the U.S. Receiving Office.
[ ]	has been transmitted by the International Bureau.
[ ]	has been transmitted by applicant on ---.

Express Mail Certification: I hereby certify that this correspondence is today being deposited with the U.S. Postal Service as "Express Mail Post Office to Addressee" Mailing Label Number EL572277018US in an envelope addressed to: Box PCT, Commissioner of Patents and Trademarks, Washington, DC 20231.

July 10, 2000

Mary M. Krinsky

2. The U.S. National Fee (35 U.S.C. 371(C)(1)) and other fees (37 CFR) are indicated below:

Claims Fees			
Claims Fee – Number Filed	No. Extra	Rate	Calculation
Total Claims* 20 minus 20	= 0	@ 18	00.00
Independent Claims: 3 minus 3	= 0	@	00.00
Multiple Dependent Claims (if applicable)	= 0	@ $260	0.00
Basic Fee – The International Search Fee, as set forth in Section 1.445(a)(2) to be paid to the US PTO acting as an International Searching Authority:	Has been paid (37 CFR 1.492(a)(2) ..... @$690		00.00
	Has not been paid (37 CFR (1.492(a)(3) ..... @$970		970.00
	Where a search report on the international has been prepared by EPO or JOP ..... @$910		00.00
		Total of above Calculations	$970.00
Small Entity - Reduction by half for filing by a Small Entity			$485.00
		Subtotal	$485.00
		Total National Fee	$485.00
Recording Assignment fee			00.00
TOTAL		**Total Fees Enclosed**	$485.00

* See attached Preliminary Amendment.

Authorization to Charge Fees. A fee transmittal and check are enclosed herewith. The Commissioner is hereby authorized to charge any additional fees by this paper and during the entire pendency of this Application to Account No. 50-0212.

3. A translation of the International Application into the English language (35 U.S.C. 371(C)(2)):

	is transmitted herewith.
xx	is not required as the application was filed in English.
	was previously transmitted by applicant on ---.

4. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)):

[ ] are transmitted herewith.

[xx] have not been transmitted, as

- [ ] no notification has been received that the International Search Authority has received the Search Copy.
- [ ] the Search Copy was received by the International Searching Authority, but the Search Report has not yet been issued.
- [xx] applicants chose not to make amendments under PCT Article 19. The date of mailing of the Search Report (from form PCT/ISA/210) is 1 June 1999.

5. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)):

- [ ] is transmitted herewith.
- [xx] is not required as the application was filed in English.
- [xx] has not been transmitted for reasons indicated at point 5 above.

6. An oath or declaration of the inventor [35 U.S.C. 371 (c)(4)] complying with 35 U.S.C. 115.

- [ ] was previously submitted by applicant on ---.
- [xx] is attached to the application.
- [ ] identifies the application and any amendments under PCT Article 19 which were transmitted as stated in points 3.b. or c. and 5.b; and states that they were reviewed by the inventor as required by 37 CFR 1.70.
- [ ] will follow.

7. An International Search Report or Declaration under PCT Article 17(2)(a):

- [xx] is transmitted herewith.
- [xx] has been transmitted by the International Bureau. The date of mailing (from form PCT/ISA/220) is 1 June 1999.

8. An Information Disclosure Statement under 37 CFR 1.97 and 1.98:

- [xx] is transmitted herewith, PTO/SB/08A and 08B (2 pages).
- [ ] will be transmitted within three months of the date of submission of requirements under 35 U.S.C. 371(c).

[ ] Also transmitted herewith is/are:

[xx] copies of citations listed (67 pages total, many double-sided).

9. Additional documents:

xx	Copy of Request (PCT/RO/101) and Chapter II Demand (PCT/IPEA/401).
xx	Copy of the International Preliminary Examination Report (PCT/IPEA/409) and Written Opinion (PCT/IPEA/408).
xx	Preliminary Amendment (37 CFR § 1.121).
xx	Small Entity Statement.
xx	Other: Fee Transmittal and check for the filing fee.

Respectfully submitted,

10 July 2000

Mary M. Krinsky

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09/600060

532 Rec'd PCT/PTC 10 JUL 2000

Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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FEE TRANSMITTAL for FY 2000

Patent fees are subject to annual revision

Small Entity payments must be supported by a small entity statement, otherwise large entity fees must be paid See Forms PTO/SB/09-12

See 37 C F R §§ 1 27 and 1 28

TOTAL AMOUNT OF PAYMENT ($) 485.00

Complete if Known	
Application Number	PCT/GB99/00070 US nat. phase entry
Filing Date	8 Jan. 1999 (internat.)
First Named Inventor	N.A. Williams
Examiner Name	
Group / Art Unit	
Attorney Docket No	CTH-03

METHOD OF PAYMENT (check one)

1. [X] The Commissioner is hereby authorized to charge indicated fees and credit any over payments to

Deposit Account Number: 50-0212

Deposit Account Name: Mary M. Krinsky

[X] Charge Any Additional Fee Required Under 37 CFR §§ 1 16 and 1.17

2. [X] Payment Enclosed:

[X] Check [ ] Money Order [ ] Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	760	201	380	Utility filing fee	$485
106	310	206	155	Design filing fee	
107	480	207	240	Plant filing fee	
108	760	208	380	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($) 485

2. EXTRA CLAIM FEES

	Extra Claims		Fee from below	=	Fee Paid
Total Claims 20	-20** = 0	X		=	0
Independent Claims 3	- 3** = 0	X		=	
Multiple Dependent				=	

**or number previously paid, if greater, For Reissues, see below

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	78	202	39	Independent claims in excess of 3
104	260	204	130	Multiple dependent claim, if not paid
109	78	209	39	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	380	216	190	Extension for reply within second month	
117	870	217	435	Extension for reply within third month	
118	1,360	218	680	Extension for reply within fourth month	
128	1,850	228	925	Extension for reply within fifth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of an appeal	
121	260	221	130	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,210	241	605	Petition to revive - unintentional	
142	1,210	242	605	Utility issue fee (or reissue)	
143	430	243	215	Design issue fee	
144	580	244	290	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	760	246	380	Filing a submission after final rejection (37 CFR § 1 129(a))	
149	760	249	380	For each additional invention to be examined (37 CFR § 1 129(b))	

Other fee (specify) _____

Other fee (specify) _____

* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Mary M. Krinsky	Registration No (Attorney/Agent)	32423	Telephone	203-773-9544
Signature	Mary M. Krinsky			Date	10 July 2000

Burden Hour Statement. This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231

CTH-03

Applicants: Neil A. Williams, Timothy R. Hirst, and John Bienenstock

Filing Date: 10 July, 2000

For: AGENT FOR TREATING ALLERGIC OR HYPERSENSITIVITY CONDITION

Verified Statement (Declaration) Claiming Small Entity
Status (37 CFR §§ 1.9(f) and 1.27(d) -- Nonprofit Organization

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization, University of Bristol, Bristol BS8 1TD, UK.

Type of Organization: University or other institution of higher education.

I hereby declare that the University identified above qualifies as a nonprofit organization as defined in 37 CFR §§ 1.9 (e) and (f) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled: **AGENT FOR TREATING ALLERGIC OR HYPERSENSITIVITY CONDITION** by inventors Neil A. Williams, Timothy R. Hirst, and John Bienenstock, described in the specification filed herewith, as the University of Bristol would qualify as a nonprofit scientific or educational organization under the statute if located in the United States.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of person signing: Peter Maxwell
Title in organization: Acting Director
Address of person signing: Research Ventures Group
University of Bristol
Senate House, Tyndall Avenue
Clinton, Bristol BS8 1TH, UK

Date: 20 June, 2000

CTH-03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of :
:
NEIL A. WILLIAMS, *et al.* :
:
Serial No.: Pending :
:
Filing Date: July 10, 2000 :

For: AGENT FOR TREATING ALLERGIC OR HYPERSENSITIVITY CONDITIONS

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Please enter the following preliminary amendments for national phase entry of PCT/GB99/00070, international filing date 8 January 1999, claiming benefit of GB serial number 9800487.2, filed 9 January 1998:

AMENDMENTS

In the claims:

Please cancel pending PCT claims 1 to 19 and replace them by adding the following new claims 20 to 39:

I hereby certify that this correspondence is today being deposited with the U.S. Postal Service as "Express Mail Post Office to Adresee" Mailing Label Number EL572277018US in an envelope addressed to: Box PCT, Commissioner of Patents and Trademarks, Washington, DC 20231.

July 10, 2000

Mary M. Krinsky
Mary M. Krinsky

20. An assay method for identifying an agent useful in the treatment of an allergic or hypersensitivity condition comprising:

(a) contacting a test agent with a ganglioside receptor, wherein the agent is not coupled to an antigen;

(b) determining whether the agent modulates a ganglioside associated activity

by measuring a change in at least one parameter selected from the group consisting of: a change in antigen specific IgE levels, a change in antigen specific T-cell reactivity, a change in IgG levels, a change in IgA levels, and any combination thereof; and

(c) identifying the useful agent by observation of modulation of ganglioside associated activity.

21. A method according to claim 20 wherein the agent binds to GM1-ganglioside receptors.

22. A method according to claim 21 wherein the agent is selected from the group consisting of Ctx, Etx, CtxB, EtxB, and mutants or derivatives thereof that bind to GM1.

23. A method according to claim 20 wherein the agent has an effect on GM1 mediated intracellular signalling events, but no GM1 binding activity.

24. A method according to claim 20 wherein the agent is capable of blocking an IgE mediated response.

25. A method according to claim 24 wherein the agent suppresses antigen-specific IgE levels.

26. A method according to claim 20 wherein the agent is capable of enhancing the production of IgG, IgA, or mixtures thereof.

27. A method according to claim 20 wherein the agent reduces the production of Th2 associated cytokines.

28. A method according to claim 27 wherein the cytokine is IL-4.

29. A method according to claim 20 wherein the agent increases the expression of cytokines which are involved in down-regulating the allergic response.

30. A method according to claim 29 wherein the cytokines are IL-10 or TGFβ.

31. A pharmaceutical composition comprising an agent identified in the assay method of claim 20.

32. A method for treating a subject for an allergic or hypersensitivity condition comprising administering to the subject an effective amount of an agent that is capable of modulating a ganglioside associated activity, wherein the agent is not coupled to an antigen, and wherein the modulation of the ganglioside associated activity affects an allergic or hypersensitivity condition, provided that the modulation is characterized by at least one change selected from the group consisting of: a change in antigen specific IgE levels, a change in antigen specific T-cell reactivity, a change in IgG levels, a change in IgA levels, and combinations thereof.

33. A method according to claim 32 wherein the agent is capable of blocking an IgE mediated response.

34. A method according to claim 32 wherein the agent exhibits GM1 binding activity, or has an effect on GM1 mediated intracellular signalling events, but no GM1 binding activity.

35. A method according to claim 34 wherein the agent is selected from the group consisting of Ctx, Etx, CtxB, EtxB, and mutants or derivatives thereof that bind to GM1.

36. A method according to claim 34 wherein the agent is EtxB.

37. A method for treating a subject for an allergic or hypersensitivity condition comprising administering to the subject an effective amount of an EtxB agent that

modifies a GM1 associated activity, wherein the agent is not coupled to an antigen, and the agent modulates at least one change selected from the group consisting of a change in antigen specific IgE levels, a change in antigen specific T-cell reactivity, a change in IgG levels, a change in IgA levels, and combinations thereof.

38. A method according to claim 37 wherein the agent binds to GM1.

39. A method according to claim 37 wherein the agent reduces levels of serum antigen-specific IgE.

REMARKS

Claims 1 to 19 were pending in this application in its published PCT format. The claims were cancelled and replaced by new claims 20 to 39 to put them into form for U.S. prosecution, replacing use claims with method claims, and to streamline the case and save considerable fees by re-writing multiple dependent claims as dependent claims. As amended, the application has a standard U.S. set of twenty claims, three of which are independent.

Newly presented independent claims 20, 32 and 37 particularly point out assay and treatment methods for identifying and using an agent useful in the treatment of an allergic or hypersensitivity condition comprising screening test agents not coupled with an antigen with a ganglioside receptor, determining whether the agent modulates gangliosideassociated activity, and identifying useful agents by observation of modulation of ganglioside associated activity. Support for the claims may be found in the specification on page 10 at lines 20 to 27, page 33 at lines 9 to 19, and page 34, lines 4 to 9.

Dependent claim 21 adds to claim 20, and claim 38, to 37, the limitation that the agent is a GM1 binding agent; support for the limitation can be found in the specification on page 12 at line 22. Claims 22 and 35 provide a list of agents set out in the specification on page 12 at lines 25 to 26. Claim 36 particularly points out a preferred embodiment wherein the agent is EtxB, as stated in the

specification on page 13 at line 2. Claim 23 points out another embodiment wherein the agents have an effect on GM1 mediated intracellular signalling events, but no GM1 binding activity, as described in the specification on page 29 at lines 23 to 25. Claim 35 combines the limitations of claims 21 and 23, as does language of the specification on page 29 at lines 21 to 25. Claims 24 and 33 point out agents that block an IgE mediated response, tracking the language of former claim 3. Claim 25 adds to claim 24, and claim 39, to 37, the limitation that antigen-specific IgE levels are suppressed; support for the limitation can be found in the specification on page 33 at lines 14 to 15. Claim 26 distinctly claims agents capable of enhancing the production of IgG and/or IgA, as described in the specification on page 34, lines 4 to 9. Claim 27 points out agents that reduce the production of Th2 associated cytokines as described in the specification on page 33 at lines 28 to 29. Claim 28 adds to claim 27 the limitation that the cytokine is IL-4 in some embodiments; support for the claim can be found in the specification on page 34 at line 1. Claims 28 and 29 point out agents that increase the expression of cytokines involved in down-regulating the allergic response, such as IL-10 or TGFβ as described in the specification on page 34 at lines 1 to 2. Claim 31 tracks the language of former claim 17. No new matter is presented.

The claimed invention provides significant new ways of treating allergic conditions and/or hypersensitivity conditions through the induction of specific immune deviation or suppression by the identification and use of agents exhibiting the properties set out in the specification. Applicants therefore request early and favorable consideration of the claims.

If the undersigned can advance the prosecution of this application in any way, the Examiner is invited to call at the number listed below.

Respectfully submitted,

MARY M. KRINSKY, Reg. No. 32423
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(203) 773-9544

AGENT FOR TREATING ALLERGIC OR HYPERSENSITIVITY CONDITION

The present invention relates to a medicament.

In particular, the present invention relates to a medicament useful to affect an allergic condition and/or a hypersensitivity condition.

More in particular, in one aspect the present invention relates to an immunological tolerance inducing agent.

More in particular, the present invention relates to such an agent optionally co-administered with a specific antigen for use in the treatment of mammalian particularly human, allergic and other hypersensitivity diseases.

When an adaptive immune response occurs in an exaggerated or inappropriate form, the term allergy or hypersensitivity is applied. Allergic or hypersensitivity reactions are the result of normally beneficial immune responses acting inappropriately to foreign antigens (usually environmental macromolecules) and sometimes cause inflammatory reactions and tissue damage. In these situations, a normally harmless environmental stimulus, called an "allergen", triggers an immune response which upon re-exposure, is re-activated to generate pathological damage. Allergies or hypersensitivities are distinguished into four types of reactions. The first three are antibody-mediated, the fourth is mediated mainly by T cells and macrophages.

In Type I Immediate Hypersensitivity/Atopic Allergy, the principal immune response to the allergen involves the production of IgE antibodies. Such disorders are by far the most prevalent in humans and are seen as principal targets for new therapeutic approaches. Although these diseases are not exclusively IgE mediated, IgE binds to cells within the tissues such as mast cells and basophils and the cross-linking of IgE on the cells surfaces by allergen invokes the release of many inflammatory mediators.

Typical examples of such diseases include asthma, allergic cough, allergic rhinitis and conjunctivitis, atopic eczema and dermatitis, urticaria, hives, insect bite allergy, dietary and certain drug allergies. In many cases, the particular allergens are known. By way of example, the principal allergen in asthma is DerP1 from house dust mite but other triggers of asthma such as pet dander antigens also exist.

Type II or antibody dependent cytotoxic hypersensitivity occurs when antibodies of a different type, usually IgG and IgM, binds to either self antigen or foreign antigen on cells and leads to phagocytosis, killer cell activity or complement mediated lysis. These types of allergies are relatively unusual but can include some allergies to drugs.

Type III hypersensitivity develops when immune complexes are formed in large quantities or cannot be cleared adequately by the reticuloendothelial system. The immune complexes usually result from the deposition of antibody, usually IgM or IgG, allergen complexes at these sites. In normal circumstances, antibody binds to allergen and is cleared by a variety of tissue cells. However, a number of factors may influence the persistence of the immune complexes and where they remain in the blood for prolonged periods, they can lodge and establish inflammation in the kidneys, skin (where they cause rashes) and joints (where they can cause a type of arthritis other than rheumatoid arthritis).

Type IV or delayed type hypersensitivity (DTH) does not involve antibody but instead the prolonged activation of T lymphocytes. These T cells are capable of secreting soluble factors causing tissue damage and enhancing the recruitment and activation of other cell types to the tissues. Incoming cells themselves contribute to the inflammation and tissue damage. DTH is most seriously manifested when antigens (for example those associated with mycobacteria tuberculosis) are trapped in a macrophage and cannot be cleared. T cells are then stimulated to elaborate cytokines which mediate a range of inflammatory responses. DTH reactions are less common than Type I reactions but are seen in graft rejection and allergic contact dermatitis

which is generally manifested as a contact sensitivity (allergy usually involving skin rash) to environmental "contact allergens" such as heavy metals.

Oral administration of antigens - such as allergens and autoantigens - has long been recognised as a method to prevent peripheral T cell responses and, in the case of autoantigens, has also been shown to prevent or delay the onset of several experimental autoimmune diseases including experimental allergic encephalomyelitis (EAE). Major problems recognised with such strategies are that it usually requires feeding of large, if not massive, doses of autoantigens and it is generally less efficient in an immune as opposed to a naive host. The latter problem has limited the therapeutic potential of this strategy. However, it has now been shown by Sun *et al* (1994 Proc Natl Acad Sci 91: 10795-10799) that oral administration of minute amounts of prototype particulate and soluble protein antigens conjugated to cholera toxin B subunit (CtxB), the nontoxic receptor-binding moiety of cholera toxin, can readily induce tolerance in the peripheral T-cell compartment and is effective not only in naive but also in systemically sensitised animals. In addition, oral administration of minute amounts of an autoantigen, myelin basic protein (MBP), coupled to CtxB can prevent EAE in Lewis rats (Sun *et al* 1996 Proc Natl Acad Sci 93: 7196-7201). Other researchers have also shown that feeding even a single dose of minute amounts (microgram) of antigens conjugated to the receptor binding nontoxic B subunit moiety of cholera toxin (CtxB) can markedly suppress systemic T cell mediated inflammatory reactions in naive as well as in experimental animals (Bergerot *et al* 1997 Proc Natl Acad Sci 94: 4610-4614).

Escherichia coli (E. coli) heat labile enterotoxin (Etx) and its closely related homologue, cholera toxin (Ctx), are examples of protein toxins which bind to glycolipid receptors on host cell surfaces. Each toxin consists of six noncovalently linked polypeptide chains, including a single A subunit (27 kDa) and five identical B subunits (11.6 kDa) which principally bind to GM1 ganglioside receptors found on the surfaces of mammalian cells (Nashar *et al* 1996 Proc Natl Acad Sci 93: 226-230). The

A subunit is responsible for toxicity possessing adenosine diphosphate (ADP) ADP-ribosyltransferase activity, whereas the B subunits (EtxB and CtxB) are non-toxic oligomers which bind and cross-link a ubiquitous cell surface glycolipid ganglioside, called GM1, thus facilitating A subunit entry into the cell.

The GM1 ganglioside receptor is a member of family of gangliosides comprising sialic acid containing glycolipids (also called glycosphingolipids) which are formed by a hydrophobic portion, the ceramide, and a hydrophilic part, that is the oligosaccharide chain. Gangliosides are defined as any ceramide oligosaccharide carrying, in addition to other sugar residues, one or more sialic residues (Oxford Dictionary of biochemistry and molecular biology. Oxford University Press. 1997. Eds Smith AD, Datta SP, Howard Smith G, Campbell PN, Bentley R and McKenzie HA). Although first described in neural tissue, several studies have shown that gangliosides are almost ubiquitous molecules expressed in all vertebrate tissues. Within cells, gangliosides are usually associated with plasma membranes, where they may act as receptors for a variety of molecules and take part in cell-to-cell interaction and in signal transduction. In addition, gangliosides are expressed in cytosol membranes like those of secretory granules of some endocrine cells such as the pancreatic islets and adrenal medulla.

Gangliosides contain in their oligosaccharide head groups one or more residues of a sialic acid which gives the polar head of the gangliosides a net negative charge at pH 7.0. The sialic acid usually found in human gangliosides is N-acetylneuraminic acid. Over 20 different types of gangliosides have been identified, differing in the number and relative positions of the hexose and sialic residues which form the basis of their classification. Nearly all of the known gangliosides have a glucose residue in glycosidic linkage with ceramide, residues of D-galactose and N-acetyl-D-galactosamine are also present.

In the ganglioside nomenclature of gangliosides, devised by Svennerholm (Biochemistry Lehninger 2nd Ed 1975 Worth Publishers Inc p 294-295), the subscript letters indicate the number of sialic groups. M is monosialo, D is disialo and T is trisialo.

One of the best studied members of the ganglioside family is the monosialosylganglioside, GM1, which has been shown to be the natural receptor for the cholera toxin. Soluble ganglioside GM1 binds to the toxin with high affinity and inactivates it (Svennerholm 1976 Adv Exp Med Biol 71: 191-204).

The chemical formula for GM1 can be represented as:

Gal β3GalNAc β4(NeuAc alpha 3)Gal β4Glc β1 Cer

where Glc is D-glucose, Gal is D-galactose, GalNAc is N-acetyl-D-galactosamine; NeuAc is N-acetylneuraminic acid, Cer is ceramide.

The chemical formula for GM1 can also be represented as

galactosyl-N-acetylgalactosaminyl {sialosyl}lactosyl ceramide

or

galactosyl-N-acetyl-galactosaminyl-(sialyl)-galactosylglusosylceramide

The x-ray crystal structures of Etx bound to lactose (Sixma *et al* 1992 Nature (London) 355: 561-564) and CtxB bound to the pentasaccharide of GM1 (Merritt *et al* 1994 Protein Sci 3: 166-175) have revealed that CtxB and EtxB bind to the terminal galactose and sialic acid moieties of GM1 which can be represented as

Galβ-1-3-3GalNAc

and that such binding does not induce any striking changes in B subunit conformation.

Furthermore the cholera toxin has been shown to demonstrate an absolute requirement for terminal galactose and internal sialic acid residues (as in GM1) with tolerance for substitution with a second internal sialic acid (as in GD1b).

Etx, like Ctx also probably binds to the terminal sugar sequence

Gal β1-3GalNAc β1-4(NeuAc alpha 2-3)Gal

where GalNAc is the N-acetylgalactosamine and NeuAc is N-acetylneuraminic acid.

In addition to binding to GM1, EtxB binds weakly to other gangliosides, including non-galactose containing GM2 and asialo-GM1 as well as galactoproteins (Nashar *et al* Immunology 1997 91: 572-578). Other researchers have shown that EtxB is capable of binding to GM1 and tolerated removal or extension of the internal sialic acid residue (as in asialo-GM1 and GD1b respectively) but not substitution of the terminal galactose of GM1 (Umesaki and Setoyama 1992 Immunology 75: 386-388).

In contrast to the poor immunogenicity of the A subunit alone, both EtxB and CtxB are exceptionally potent immunogens and their respective holotoxins, Etx and Ctx, are known to be exceptionally potent adjuvants when given orally in combination with unrelated antigens (Ruedl *et al* 1996 Vaccine 14: 792-798; Nashar *et al* 1993 Vaccine 11: 235; Nashar and Hirst 1995 Vaccine 13: 803; Elson and Ealding 1984 J Immunol 133: 2892; Lycke and Holmgren 1986 Immunology 59: 301). Because of their remarkable immunogenicity, both EtxB and CtxB have been used as carriers for other epitopes and antigens (Nashar *et al* 1993 *ibid*) and have been used as components of vaccines against cholera and *E.coli* diarrhoea (Jetborn *et al* 1992 Vaccine 10: 130).

The ability of the B subunit of Ctx and Etx to interact with receptors present on mammalian cells has been shown to exert modulatory effects on the function of those cells. It is known that cells of the immune system are differentially affected following such interaction. In particular, WO 95/020045 discloses that EtxB binds to GM1 ganglioside receptors which are found on the surfaces of mammalian cells and that this binding induces differential effects on lymphocyte populations including a specific depletion of CD8+ T cells and an associated activation of B cells. These effects are absent when a mutant EtxB protein lacking GM1 binding activity is employed. These observations have led to the use of agents capable of binding to GM1 in the prevention and treatment of autoimmune disease, transplant rejection and graft versus host disease (GVHD). These studies suggest that agents that bind to GM1 or mimic binding to GM1 promote the induction of immunological tolerance.

Researchers have shown that a state of immunological unresponsiveness, also known as "immunological or oral tolerance", can be induced by the oral administration of dietary protein antigens. (Sun *et al* 1994 *ibid*; Sun *et al* 1996 *ibid*; Bergerot *et al* 1997 *ibid*). The inhalation of antigens can also induce a state of specific immunological unresponsiveness or "nasal tolerance". Thus, systemic immunological tolerance can be induced when antigen is administered orally or nasally by a mucosal route. WO 95/01301 discloses an immunological tolerance-inducing agent comprising a mucosa-binding agent linked to a specific tolerogen. WO95/10301 also includes mention of the treatment of allergy using a mucosa binding agent coupled to an allergen. Other researchers such as Tamura *et al* (1997 Vaccine 15: 225-229) have taken directly the protocol of WO 95/10301 and tested its efficacy in preventing allergy in a murine model of Type I allergy. They reported a significant lowering of IgE levels which are a strong predictor of efficacy and they cite data, following administration of EtxB coupled to ovalbumin (the results were not included), which shows that EtxB was not effective once IgE levels are established. It has also been shown that orally administered Ctx and Etx can act on several humoral and cellular immune responses not only at the gastrointestinal tract, but also in distant mucosal effector sites such as

the respiratory tract. These data suggest that these mucosal adjuvants have a potential use in oral immunisation strategies to improve the local immune responses in remote mucosal tissues, in accordance with the concept of a common mucosal immune system (Bienenstock J 1974 The physiology of the local immune system and the gastrointestinal tract. In: Progress in Immunology II, vol 4: clinical aspects, I.L. Brent, J.Holborrow, Eds. Amsterdam, North Holland, pp197-207; Ruedl *et al* 1996 *ibid*; Umesaki 1992 *ibid*; Czerkinsky and Holmgren (1994 Cell Mol Biol 40: 37-44).

The induction of immunological tolerance may include a number of different mechanisms which may be summarised as follows:

(i) a process whereby antigen reactive cells are removed through triggering them to commit suicide (apoptosis);

(ii) an induction of anergy or the long term inactivation of the antigen reactive cells;

(iii) immune deviation of the antigen reactive cells away from the production of pathological responses;

(iv) suppression of the antigen reactive cells or their regulation by specific factors or regulatory cells

In the treatment of allergy, it is possible that the induction of any of these mechanisms may be useful. However, while the deletion of antigen reactive cells and/or the induction of anergy are useful strategies once the precise allergens are known, invoking these mechanims will usually silence only those cells which respond to the allergen which was given in the treatment regime. On the other hand, the implementation of immune deviation or suppression strategies has the advantage of potential regulation of responses to antigens which are involved in the condition but

were not part of the treatment. This phenomenon, known as "bystander suppression" allows the "spread" of tolerance to other antigens (such as allergens) in the target tissues through either the possible secretion of non-antigen specific suppressor molecules or through suppressive cellular interactions in that tissue as a result of the interaction between the antigen specific cells and the specific immunising antigen. In this way, as long as at least one of the antigens involved in the disorder is known, the condition may be treated even if there are other antigens implicated as well. Thus, the goal of a good treatment is the induction of a specific immune deviation or suppression.

Nashar and co-workers (Proc Natl Acad Sci 1996 93: 223-226; Int Immunol 1996 8: 731-736; Immunol 1997 91: 572-578) have demonstrated that the administration of EtxB and other homologues can modulate the immune response away from the production of Th1 cytokines such as IFNγ and interleukin 2 (IL-2) and towards the secretion of Th2 cytokines such as IL-4, IL-10 and IL-13. IFNγ is the classical Th1 cytokine, IL-4 is the classical Th2 cytokine. This "immune deviation" is the basis of the disclosure in WO 97/02045 and has been shown to be effective in the treatment of autoimmune diseases. The experimental results in WO 97/02045 would suggest that GM1 binding agents would not find use in the treatment of allergic conditions and/or hypersensitivity conditions since such conditions involve IgE, the production of which is generally accepted to be promoted by IL-4 and down regulated by IFNγ.

The present invention now seeks to provide new ways of treating allergic conditions and/or hypersensitivity conditions through the induction of a specific immune deviation or suppression.

According to a first aspect of the present invention, there is provided the use of an agent in the manufacture of a medicament to affect an allergic condition and/or a hypersensitivity condition; wherein the agent is capable of modulating a ganglioside associated activity; wherein the agent is not coupled to an antigen; and wherein the modulation of the ganglioside associated activity affects an allergic condition and/or a hypersensitivity condition.

According to a second aspect of the present invention, there is provided the use of an agent in the manufacture of a medicament to affect an allergic condition and/or a hypersensitivity condition; wherein the agent is capable of modulating a GM1 associated activity; wherein the agent is not coupled to an antigen; and wherein the modulation of the GM1 associated activity affects an allergic condition and/or a hypersensitivity condition.

According to a third aspect of the present invention, there is provided an agent according to the present invention capable of blocking an IgE mediated response.

Preferably, the agent is capable *in vivo* of blocking an IgE mediated response.

According to a fourth aspect of the present invention, there is provided an assay method for identifying an agent according to the present invention capable of affecting an allergic condition and/or a hypersensitivity condition; wherein the assay method comprises: (a) contacting an agent with a ganglioside; (b) determining whether the agent modulates a ganglioside associated activity; such that the modulation of the ganglioside associated activity is indicative that the agent may be capable of affecting an allergic condition and/or a hypersensitivity condition; and wherein the agent is not coupled to an antigen.

According to a fifth aspect of the present invention, there is provided an assay method according to the present invention wherein the assay is an assay to screen for an agent useful in the prevention and/or treatment of an allergic condition and/or a hypersensitivity condition.

According to a sixth aspect of the present invention, there is provided a process comprising the steps of: (a) performing the assay according to the present invention; (b) identifying one or more agents capable of modulating a ganglioside associated activity; and (c) preparing a quantity of those one or more agents.

According to a seventh aspect of the present invention, there is provided a process comprising the steps of: (a) performing the assay according to the present invention; (b) identifying one or more agents capable of modulating a ganglioside associated activity; and (c) preparing a pharmaceutical composition comprising those one or more identified agents.

According to an eighth aspect of the present invention, there is provided a process comprising the steps of: (a) performing the assay according to the present invention; (b) identifying one or more agents capable of modulating a ganglioside associated activity; and (c) modifying one or more identified agents capable of modulating a ganglioside associated activity; and (d) preparing a pharmaceutical composition comprising those one or more modified agents.

According to a ninth aspect of the present invention, there is provided an agent identified by the process of the present invention.

Preferably the agent identified had not previously been known to affect an allergic condition and/or a hypersensitivity condition through modulation of a ganglioside associated activity.

According to a tenth aspect of the present invention, there is provided a method of affecting an allergic condition and/or a hypersensitivity condition with one or more agents; wherein the agent is capable of modulating a ganglioside associated activity in an *in vitro* assay method; and wherein the *in vitro* assay method is the assay method defined in the present invention.

Preferably there is provided a method of affecting *in vivo* an allergic condition and/or a hypersensitivity condition with one or more agents; wherein the agent is capable of modulating a ganglioside associated activity in an *in vitro* assay method; and wherein the *in vitro* assay method is the assay method defined in the present invention.

According to a eleventh aspect of the present invention, there is provided an agent according to the present invention for use as a pharmaceutical.

.According to a twelfth aspect of the present invention, there is provided the use of an agent according to the present invention in the manufacture of a medicament to affect an allergic condition and/or a hypersensitivity condition.

According to a thirteenth aspect of the present invention, there is provided a pharmaceutical composition comprising or prepared from an agent according to the present invention.

Preferably the agent is a GM1 binding agent.

Preferably, the agent capable of modulating a ganglioside associated activity is selected from a group consisting of Ctx, Etx, CtxB and EtxB.

Preferably, the agent capable of modulating a ganglioside associated activity is capable of blocking an IgE mediated response in a subject with an allergic condition and/or a hypersensitivity condition.

Preferably the subject is a human - e.g. a human patient.

Preferably the agent is EtxB.

In a particularly preferred embodiment the agent is the wild type EtxB.

Alternatively, preferably the agent is either a mutant of EtxB which is capable of modulating a ganglioside associated activity or other equivalent proteins thereof.

Preferably the agent(s) is/are non-toxic.

Preferably the agent is CtxB and mutants thereof which are capable of modulating a ganglioside associated activity.

Preferably the ganglioside is a GM1 ganglioside receptor.

Preferably the agent capable of modulating a ganglioside associated activity is capable of cross-linking GM1 ganglioside receptors.

Preferably EtxB is one such agent which is capable of cross-linking GM1 ganglioside receptors by virtue of its pentameric form.

Preferably the medicament is used for the treatment or prophylaxis of a Type I allergic and/or a Type IV hypersensitivity condition such as contact hypersensitivity.

Preferably the medicament includes one or more antigens which are optionally co-administered with antigen.

Preferably the agent may be administered to a mammal with or without co-administration of an antigen.

Preferably the mammal is a human - e.g. a human patient.

In accordance with the present invention we have surprisingly found that the use of agents capable of modulating a ganglioside associated activity, when given alone or when co-administered with suitable antigens, can be used as an effective treatment for allergic and/or hypersensitivity conditions. Previous workers have either not attempted to find a mechanism (Sun *et al* 1996 *ibid*) or have argued that agents capable of modulating a ganglioside associated activity, such as EtxB and CtxB, cause a Th1 to Th2 switch in the immune response to antigen (WO97/02045). Since allergic conditions are known in the art to be promoted by Th2 responses, then the previous findings suggest that such agents would either be ineffective in treating allergies or may even worsen them.

We have surprisingly found that while EtxB and CtxB promote some aspects of Th2-associated responses, in some cases, they may not stimulate the production of the key factor in triggering allergy, IgE. Thus allergic conditions and/or hypersensitivity conditions can be treated with an agent capable of modulating a ganglioside associated activity, for instance, which is not coupled with an antigen.

Significantly, the linkage of the components was not found to be necessary. Furthermore, our findings indicate that the mechanisms of protection against allergic conditions and/or hypersensitivity conditions may include, though not be limited to either the suppression of antigen specific IgE secretion and/or the upregulated production of non-inflammatory antigen specific antibody isotypes (particularly IgG and IgA).

Thus, the present invention is advantageous because allergic conditions and/or hypersensitivity conditions can be treated with an agent capable of modulating a ganglioside associated activity which is optionally co-administered with an antigen.

The term "ganglioside" as used with respect to the present invention include its normal definition in the art (such as that defined above) as well as active fragments thereof.

The ganglioside can be made synthetically or isolated from natural sources. Alternatively, it can be obtained from commercial sources.

The term "ganglioside associated activity" includes any one or more of modulating or immunomodulating a ganglioside receptor, modulating any signalling event prior to, during or subsequent to ganglioside receptor binding.

The term "Ctx" refers to the cholera toxin and CtxB refers to the B subunit of the cholera toxin. In other texts, these may sometimes be identified as CT or Ct or CTB or CtB respectively.

The term "Etx" herein means the *E. coli* heat labile enterotoxin and EtxB is the B subunit of Etx. In other texts, these may sometimes be identified as LT or Lt and LTB or LtB respectively.

The term "adjuvant" includes a substance that enhances an immune response to an antigen.

The term "mucosal adjuvant" includes an agent which is delivered mucosally with an unrelated antigen, such that the agent is capable of facilitating a mucosal immune response to the unrelated antigen. In this way, the agent acts as a so-called mucosal adjuvant.

The term "mucosal surfaces" includes but is not limited to oral, sublingual, intranasal, vaginal, rectal, salivary, intestinal and conjunctival surfaces.

The term "mucosal membrane" and/or "mucosal tissue" includes but is not limited to the intestine, the lung, the mouth, the genital tract, the nose and the eye.

A "vaccine carrier" includes a carrier of relevant antigens (Szostak *et al* 1996 J Biotechnol 44: 161-170)

The term "mucosal immunogen" includes an agent administerable by a mucosal route that has the capability to evoke local and/or systemic antibody production and/or cell mediated immune reactions and/or delayed type hypersensitivity reactions.

A "hapten" means a small molecule which can act as an epitope but is incapable by itself of eliciting an antibody response.

The term "immunological or oral tolerance" means a reduction in immunological reactivity of a host towards a specific tolerated antigen(s). Immunological or oral tolerance may not mean a complete suppression of the immune response to a particular antigen but it is a form or tolerance also known as "immune deviation" or "split tolerance".

The term "immune deviation" or "split tolerance" can be used to describe the likely preservation of local IgA responses with the retention of some IgG responses but with the down regulation of delayed hypersensitivity and/or IgE responses.

The term "tolerance" means a state of specific immunological unresponsiveness.

A "tolerogen" means a tolerated antigen.

The term "autoimmunity" is used to describe the process by which the body generates an immune response to self-antigens.

The term "agent capable of modulating a ganglioside associated activity" can be used to describe any agent which acts as an immunomodulator through interacting with a ganglioside.

The term "GM1 binding agent" includes any agent which acts as an immunomodulator through interacting with a GM1 ganglioside receptor.

The term "immunomodulator" includes any agent that alters the extent of the immune response to an antigen, by altering the antigenicity of the antigen or by altering in a nonspecific manner the specific reactivity or the nonspecific effector associated mechanisms of the host.

The term "administered" includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno-associated viral (AAV) vectors, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

The term "co-administered" means that the site and time of administration of each of the agent and the antigen are such that the necessary modulation of the immune system is achieved. Thus, whilst the agent and the antigen may be administered at the same moment in time and at the same site, there may be advantages in administering the agent at a different time and to a different site from the antigen. The agent and antigen may even be delivered in the same delivery vehicle (such as Macrosol™ - see WO95/13795 and WO96/14871) - but with the proviso that the agent and the antigen are uncoupled.

The term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

The term "systemic immunisation" means the introduction of an antigen into a non-mucosal tissue such as the skin or the blood.

The term "self antigens" means components derived from host tissues.

The term "target interaction components" includes but is not limited to an agent capable of modulating a ganglioside associated activity, a ganglioside and/or an antigen.

The term "coupled" - which is synonymous with the term "linked" - means the linkage of the agent with the antigen - which includes but is not limited to direct linkage (such as by an ionic or covalent bond) or indirect linkage by the provision of suitable spacer groups.

The term "uncoupled" - which is synonymous with the term "unlinked" - means that the agent is not coupled to the antigen.

However, in accordance with the present invention, the agent and/or antigen can be coupled to another entity.

The term "affect" includes modulation, such as treatment, prevention, suppression, alleviation, restoration or other alteration of pre-existing condition and/or to potentially affect a future condition, as well as any combination thereof.

An "antigen" means an agent which, when introduced into an immunocompetent animal, stimulates the production of a specific antibody or antibodies that can combine with the agent. The antigen may be a pure substance, a mixture of substance or soluble or particulate material (including cells or cell fragments). In this sense, the term includes any suitable antigenic determinant, auto-antigen, self-antigen, tolerogen, allergen, hapten, and immunogen, or parts thereof, as well as any combination thereof, and these terms are used interchangeably throughout the text.

An "allergen" includes any antigen that stimulates an allergic reaction, inducing a Type I hypersensitivity reaction.

Examples of common allergen sources are outlined in the Table below.

Group	Examples of Allergens
Airborne	
grass pollens	ragweed, rye, couch, wild oat, timothy, Bermuda, Kentucky blue, mugwort
tree pollens	alder, birch, hazel, beech, Cupressae, oak,olive
moulds	*Aspergillus* spp., *Cladosporium* spp., *Alternaria* spp., Basidospores, Ascomycetes
cereal grains	wheat, rye, oat
animal dander and urine	cat, dog, horse, rabbit, guinea pig, hamster
bird feathers	budgerigar, parrot, pigeon, duck, chicken
house dust mite	*Dermatophagoides pteronyssinus, D.farinae, Euroglyphus maynei*
insects	cockroach, fly, locust, midge
Oral	
foods	seafood, legumes, peanuts, nuts, cereals, dairy products, eggs, fruits, tomatoes, mushrooms, alcoholic beverages, coffee, chocolate
drugs	penicillins, sulphonamides and other antibiotics, sulphasalazine, carbamazepine
Injected	
insects	bee and wasp stings, ant and mosquito bites
drugs	blood products, sera, vaccines, contrast media, drugs (including anti-asthma drugs and antibiotics)

The term "allergic condition" includes but is not limited to asthma, allergic cough, allergic rhinitis and conjunctivitis, atopic eczema and dermatitis, uticaria, hives, insect bite allergy, dietary allergy (peanut, fish milk, wheat etc) and drug allergies

The term "hypersensitivity condition" includes but is not limited to conditions such as contact hypersensitivity induced by plant poison ivy.

The term "agent" includes entities capable of modulating a ganglioside associated activity. The agent can be one or more of an inorganic or organic chemical, as well as combinations thereof. By way of example the agent can be a polypeptide as well as a variant/homologue/derivative/fragment thereof so long as they retain the required immunomodulatory activity. It also includes mimics and equivalents and mutants thereof. Other agents for the treatment of allergic conditions or hypersensitivity conditions include antibodies to the target interaction components. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, fragments produced by a Fab expression library and specifically designed humanised monoclonal antibodies.

Agents capable of modulating a ganglioside associated activity may be designed and produced as outlined above, by methods which are known in the art. By way of example, when the agent of the invention is a protein such as the EtxB subunit or the CtxB subunit, it may be produced, for use in all aspects of this invention by a method in which the gene or genes coding for the specific polypeptide chain (or chains) from which the protein is formed, is inserted into a suitable vector and then used to transfect a suitable host. For example, the gene coding for the polypeptide chain from which the EtxB assemble may be inserted into, for example, plasmid pMM68, which is then used to transfect host cells, such as *Vibrio sp.60.* The protein is purified and isolated in a manner known *per se*. Mutant genes expressing active mutant EtxB protein may then be produced by known methods from the wild type gene.

Where a target interaction component is a protein, procedures well known in the art may be used for the production of antibodies to that component.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc. may be immunized by injection with the target interaction component or any derivative or homologue thereof or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to

increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminium hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, and dinitrophenol. BCG (*Bacilli Calmette-Guerin*) and *Corynebacterium parvum* are potentially useful human adjuvants.

Where a target interaction component is a protein, monoclonal antibodies to that component may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique originally described by Koehler and Milstein (1975 Nature 256:495-497), the human B-cell hybridoma technique (Kosbor *et al* (1983) Immunol Today 4:72; Cote *et al* (1983) Proc Natl Acad Sci 80:2026-2030) and the EBV-hybridoma technique (Cole *et al* (1985) Monoclonal Antibodies and Cancer Therapy, Alan R Liss Inc, pp 77-96). In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison *et al* (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger *et al* (1984) Nature 312:604-608; Takeda *et al* (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies (US Patent No. 4,946,779) can be adapted to produce target interaction component specific single chain antibodies.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al* (1989, Proc Natl Acad Sci 86: 3833-3837), and Winter G and Milstein C (1991; Nature 349:293-299).

Antibody fragments which contain specific binding sites for a target interaction components may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of

the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD *et al* (1989) Science 256:1275-128 1).

The target interaction components of the present invention or a derivative or homologue thereof and/or a cell line that expresses the target interaction components of the present invention or a derivative or homologue thereof may be used to screen for antibodies, peptides, or other agent, such as organic or inorganic molecules, that act as modulators of the target interaction, thereby identifying a therapeutic agent capable of modulating the target interaction. For example, antibodies capable of modulating the target interaction may be identified.

Alternatively, screening of peptide libraries or organic libraries made by combinatorial chemistry with recombinantly expressed target interaction components or a derivative or homologue thereof or cell lines expressing the target interaction components or a derivative or homologue thereof may be useful for identification of therapeutic agents that function by modulating the target interaction. Synthetic compounds, natural products, and other sources of potentially biologically active materials can be screened in a number of ways deemed to be routine to those of skill in the art.

A target interaction component polypeptide, its immunogenic fragments or oligopeptides thereof can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The polypeptide employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The abolition of activity or the formation of binding complexes between the target interaction component and the agent being tested may be measured.

Alternatively, phage display can be employed in the identification of candidate agents which affect the target interaction components.

Phage display is a protocol of molecular screening which utilises recombinant bacteriophage. The technology involves transforming bacteriophage with a gene that encodes an appropriate ligand (in this case a candidate agent) capable of reacting with a target interaction component (or a derivative or homologue thereof) or the nucleotide sequence (or a derivative or homologue thereof) encoding same. The transformed bacteriophage (which preferably is tethered to a solid support) expresses the appropriate ligand (such as the candidate agent) and displays it on their phage coat. The entity or entities (such as cells) bearing the target molecules which recognises the candidate agent are isolated and amplified. The successful candidate agents are then characterised. Phage display has advantages over standard affinity ligand screening technologies. The phage surface displays the candidate agent in a three dimensional configuration, more closely resembling its naturally occuring conformation. This allows for more specific and higher affinity binding for screening purposes.

Accordingly, the present invention provides a method for screening a plurality of agents for specific binding affinity with the target interaction component or a derivative or homologue thereof comprising providing a plurality of agents; combining the target interaction components or a derivative or homologue thereof with each of a plurality of agents for a time sufficient to allow binding under suitable conditions; and detecting binding of the target interaction components, or a derivative or homologue thereof to each of the plurality of agents, thereby identifying the agent or agents which specifically bind the target interaction components. In such an assay, the plurality of agents may be produced by combinatorial chemistry techniques known to those of skill in the art.

Another technique for screening provides for high throughput screening of agents having suitable binding affinity to the target interaction components polypeptides and is based upon the method described in detail in WO 84/03564. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test agents are reacted with the target interaction component fragments and washed. A bound target interaction component is then detected - such as by appropriately adapting methods well known in the art. A purified target interaction component can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

The present invention also provides a pharmaceutical composition for treating a subject in need of same comprising administering a therapeutically effective amount of an agent capable of modulating a ganglioside associated activity and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

The pharmaceutical compositions may be for human or animal usage and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier, excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

The pharmaceutical composition may be formulated together with an appropriate antigen.

Alternatively, a kit may be provided comprising separate compositions for each of the therapeutic agent and the antigen.

In some embodiments of the present invention, the pharmaceutical compositions will comprise one or more of: an agent that has been screened by an assay of the present invention; wherein the agent is capable of modulating a ganglioside associated activity

The present invention also relates to pharmaceutical compositions comprising effective amounts of antigen in admixture with a pharmaceutically acceptable diluent, carrier, excipient or adjuvant (including combinations thereof).

The present invention also provides a method of treating a subject in need of same comprising administering to said subject an effective amount of the pharmaceutical composition of the present invention.

The present invention relates to pharmaceutical compositions which may comprise all or portions of the target interaction components alone or in combination with at least one other agent, such as a stabilizing compound, and may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water.

There may be different composition/formulation requirements dependent on the different delivery systems.

The pharmaceutical composition of the present invention may be formulated to be delivered by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestable solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by both routes.

Where the agent is delivered mucosally through the gastrointestinal mucosa, it is preferably stable during transit though the gastrointestinal tract; for example, it is preferably resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile.

Typically, a physician will determine the actual dosage which will be most suitable for a subject and it will vary with the age, weight and response of the particular subject. While a single dose of the agent and the antigenic determinant may be safisfactory, multiple doses are contemplated within the scope of the invention.

Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intracavernosally, intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

There may be different delivery requirements dependent on the different composition/formulation systems.

Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of the agent to the targeted tissue and/or cell population. Methods which are well known to those

skilled in the art can be used to construct recombinant vectors containing the agent. Alternatively, the agent can be delivered to target cells in liposomes.

By way of example, the controlled release of antigens on mucosal surfaces using biodegradable polymer microspheres may help to target antigens and reduce the numbers of doses required for primary immunisation (Gupta and Siber 1995 Vaccine 13: 1263-1276).

Encapsulation of vaccines in biodegradable microspheres provides excellent mucosal immunogens. Recombinant Norwalk Virus-like (rNV) particles may also be used for mucosal antigen delivery (Ball *et al* 1996 Arch Virol Suppl 12: 243-249).

Viral Like Particles (VLPs) have been utilised as vaccine delivery system for multiple immunogens including B and T cell epitopes (Roy 1996 Intervirology 39: 62-71).

One preferred method of oral delivery uses formations as described in WO95/13795, WO96/17593 and WO96/17594. These formulations allow macromolecules such as proteins to be solubilised in "oils" for oral delivery. Such formulations therefore allow delivery of the macromolecules to mucosal surfaces in the gut.

In a further approach, again when the therapeutic agent is a protein, it is possible to deliver such proteins by means of a bacterial delivery system such as that described in WO 93/17117. This system utilises the bacterium *Lactococcus lactis* to deliver proteins, for instance orally or indeed by other mucosol routes such as nasally.

In summary, the present invention provides the use of an agent in the manufacture of a medicament to affect an allergic condition and/or a hypersensitive condition; wherein the agent is capable of modulating a ganglioside associated activity wherein the agent is not coupled with an antigen.

In another broad aspect, the present invention provides an immunological tolerance inducing agent comprising an agent capable of modulating a ganglioside associated activity which is not coupled to an antigen.

Other aspects of the present invention are now presented below by way of numbered paragraphs, which include:

1. The use of an agent having GM1 binding activity, or an agent having an effect on GM1 mediated intracellular signalling events, but no GM1 binding activity, in the preparation of a medicament to treat an allergic or other hypersensitive condition, with the proviso that said agent is not coupled with an allergen and/or an antigen.

2. The use as defined in paragraph 1 wherein the agent is a GM1 binding agent such as Ctx, Etx, CtxB or EtxB or a mutant form or derivative thereof.

3. The use as defined in paragraph 1 and paragraph 2 wherein the medicament is for the prophylaxis or treatment of asthma, allergic cough, allergic rhinitis, conjunctivitis, atopic eczema, dermatitis, uticaria, hives, insect bite allergy, dietary allergy (peanut, fish, milk, wheat etc), drug allergies or contact and other hypersensitivities.

4. A method for the treatment or prophylaxis of an allergic or other hypersensitive condition which comprises administering to a subject an effective amount of an agent having GM1 binding activity, or an agent having an effect on GM1 mediated intracellular signalling events, but no GM1 binding activity, with the proviso that said agent is not coupled with an allergen and/or an antigen.

5. A method as defined in paragraph 4 wherein the agent is a GM1 binding agent such as Ctx, Etx, CtxB or EtxB or a mutant form or derivative thereof.

6. A method as defined in paragraph 4 or paragraph 5 wherein the method is for the prophylaxis or treatment of asthma, allergic cough, allergic rhinitis, conjunctivitis, atopic eczema, dermatitis, uticaria, hives, insect bite allergy, dietary allergy (peanut, fish, milk, wheat etc), drug allergies or contact hypersensitivity.

7. A pharmaceutical composition for the treatment of a human allergic and/or hypersensitivity disease comprising

(i) an agent having GM1 binding activity; or

(ii) an agent having an effect on GM1 mediated intracellular signalling events, but no GM1 binding activity;

with the proviso that the agent is not coupled with an allergen/antigen; and a pharmaceutically acceptable carrier or diluent therefor.

8. A product comprising an agent having GM1 binding activity, or an agent having an effect on GM1 mediated intracellular signalling events, but no GM1 binding activity, in the preparation of a medicament to treat an allergic or other hypersensitive condition, with the proviso that said agent is not coupled with an allergen and/or an antigen, and at least one antigen/allergen as a combined preparation for simultaneous, separate or sequential use.

The present invention will now be described only by way of example.

EXAMPLES

Screens for Agents capable of modulating ganglioside associated activity

Agents capable of modulating ganglioside associated activity are tested by any one of a variety of methods.

Examples of such methods include, but are not limited to the following methods:

1. Binding to a ganglioside receptor, such as GM1, is determined by using purified GM1 to coat microtiter plates. Following blocking of further non-specific protein binding to the plate, the agent under investigation is applied to the plate and allowed to interact prior to washing and detection with specific antibodies to said agent. Conjugation of the antibodies either directly or indirectly to an enzyme or radiolabel allows subsequent quantification of binding either using colormetric or radioactivity based methods (ELISA or RIA respectively).

2. The pentasaccharide moiety of a ganglioside, such as GM1, is bound to a suitable column matrix in order to allow standard affinity chromatography to be performed. Removal of known compounds applied to the column from the diluent are used as evidence for binding activity. Alternatively, where mixtures of compounds are applied to the column, elution and subsequent analysis allows the properties of the agent capable of modulating ganglioside associated activity to be determined.

Protein analysis includes peptide sequencing and tryptic digest mapping followed by comparisons with available databases. If eluted proteins cannot be identified in this way, then standard biochemical analysis, such as, for example, mass determination by laser desorption mass spectrometry is used to further characterise the compound. Non-proteins eluted from GM1-affinity columns are analysed by HPLC and mass spectrometry of single homogenous peaks.

3. The ability to bind to gangliosides, such as GM1, and the precise affinity of the interaction may be determined using plasmon surface resonance as previously reported [Kuziemko *et al* (1996) Biochem 35:6375-6384].

Evaluation of identified agents

The identification of agents capable of modulating ganglioside associated activity such that the modulation of the ganglioside associated activity affects an allergic condition and/or a hypersensitivity condition is determined as follows:

Laboratory animals are stimulated to produce antigen-specific IgE by methods well known in the art. By way of example, mice are challenged with alum precipitated soluble protein antigen (e.g. ovalbumin or allergens known to be involved in human allergic diseases such as ragweed or house dust mite antigens) either subcutaneously or intraperitoneally.

In the unmanipulated animal, this procedure routinely leads to the production of antigen-specific IgE which is easily detected in the serum, by standard ELISAs, using the antigen to coat suitable microtiter plates. Serum from the immunised mice is applied to the plates after non-specific protein binding has been blocked and the presence of IgE is determined using widely available labelled antibodies specific for murine IgE.

In order to screen agents for their capability to prevent or treat allergy, agents capable of modulating ganglioside associated activity are administered to mice either in the presence or absence of the challenge antigen at a range of doses, and by a variety of routes. Although the oral route is the preferred method of administration, delivery can be by other mucosal surfaces or parenterally. The frequency of such administration as well as the timing of repetitive dosing is also investigated. Such intervention strategies are utilised either prior to the IgE inducing antigen challenge (prophylaxis) or after the

IgE inducing antigen challenge (treatment). Antigen challenge can be either with (i) the antigen used as part of the prophylactic or treatment protocol; (ii) an unrelated antigen or (iii) a mixture of the challenge and unrelated antigen in order to test the specificity of the response and the induction of bystander suppression respectively.

Efficacy is determined in a variety of ways and is manifested as a number of different outcomes.

1. Antigen-specific IgE levels. Measurement of serum IgE by specific ELISA (as described) is used to determine whether prophylactic or treatment protocols are capable of reducing levels of serum antigen-specific IgE. Other methods known in the art for the determination of IgE response are used either as alternatives to ELISA or in order to provide complementary data. Such methods include the so-called "Ussing Chamber test" or "passive cutaneous anaphylaxis" assay. A reduction in specific IgE, as determined by any of these assays, is a strong marker of potential clinical efficacy.

2. Antigen specific T-cell reactivity. The responses of T-cells, derived from secondary lymphoid organs of the treated animals to the challenge antigen, is investigated using established methodology. Cell suspensions are prepared and cultured, in the presence or absence of the challenge antigen. At appropriate time intervals after the initiation of the cultures, samples are assessed for cell proliferation and cytokine production.

Cytokines are measured by specific capture ELISA, by intracellular staining followed by cytometric analysis, by RT-PCR or by other established procedures. Comparison of cell proliferation and cytokine production, in the presence of antigen as opposed to its absence, provides in each case a measure of that part of the response which is specific to the challenge antigen. Evidence of efficacy of prophylactic or treatment protocols is demonstrated by a reduction in the production of Th2 associated cytokines (in

particular IL-4) or by an increased expression of cytokines which are involved in down-regulating the allergic response (for example, IL-10 or TGFβ).

3. IgG and IgA levels. Protocols which do not reduce the levels of antigen specific IgE can still be considered as potentially effective in the event that they are also able to enhance the production of other non-allergy associated antibody isotopes. Thus investigation of serum and mucosal secretions from animals which have been either untreated or given agents under investigation as part of prophylactic or treatment protocols for the presence of IgG and IgA are also carried out. Standard antigen specific ELISA assays (as described) utilising detecting antibodies specific for IgG and specific subclass thereof, and IgA are used for this purpose. Enhanced production of secreted or serum IgG or IgA antibodies indicate efficacy since such antibodies can be expected to prevent an allergen from cross-linking IgE bound to mast cells, basophils and cosinophils or limit the uptake of antigen across the mucosal epithelium and hence prevent the subsequent allergic inflammatory response.

Enzyme Linked Immunosorbent Assays (ELISAs)

Binding of EtxB or EtxB (G33D) to GM1 is examined by a GM1-ELISA (Amin, T., & Hirst, T.R. (1994) Prot. Express. and Purif. 5, 198-204).

Sera and gut secretions are examined for the presence of anti-B subunit IgG and IgA antibodies by ELISAs in which samples are applied to microtitre plates (Immulon I, Dynateck, USA) coated with 5μg/ml of either EtxB or EtxB (G33D) in PBS. Anti-B subunits IgA antibodies in gut secretion supernatants are extrapolated from a standard curve made by coating 2 rows of wells on each plate with 1μg/ml rabbit anti-mouse IgA (α chain specific; Zymed Lab, USA) in PBS followed by addition of 1μg/ml of mouse myeloma IgA (MOPC 315, Sigma, USA). To measure total IgA, wells are coated with rabbit anti-mouse IgA followed by addition of gut secretion supernatants. All samples are serially diluted. Goat anti-mouse IgG (Fc fragment specific; Jackson

Lab., USA) or goat anti-mouse IgA (a chain specific; Sigma) peroxidase conjugate are diluted and added to all wells. The anti-B subunit IgG titer, giving an $A_{450nm} \geq 0.2$, is determined. The IgA anti-B subunit response for each of EtxB and EtxB (G33D) in gut secretions is calculated as "IgA specific activity" [mean IgA anti-B subunit (μg/ml) /total IgA (μg/ml)].

A known ELISA method for measuring cytokine levels of IL-2, IL-4, IL-5, IL-10 and IFN-γ is used. Briefly, microtiter plates are coated with rat antibodies to mouse IL-2, IL-4, IL-5, IL-10 and IFN-γ. Plates are blocked with 2% (w/v) bovine serum albumin. Supernatants from culture medium are added to wells and diluted down. One row on each plate for each cytokine contains a standard amount of recombinant cytokines. Plates are then incubated with 0.5μg/ml of biotinylated anti-cytokine monoclonal antibodies followed by addition of avidine-peroxidase and 3,3', 5,5' - Tetramethylbenzidene (TMB) substrate and read at A_{450nm}.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. The use of an agent in the manufacture of a medicament to affect an allergic condition and/or a hypersensitivity condition;

wherein the agent is capable of modulating a ganglioside associated activity;

wherein the agent is not coupled to an antigen; and

wherein the modulation of the ganglioside associated activity affects an allergic condition and/or a hypersensitivity condition.

2. The use of an agent in the manufacture of a medicament to affect an allergic condition and/or a hypersensitivity condition;

wherein the agent is capable of modulating a GM1 associated activity;

wherein the agent is not coupled to an antigen; and

wherein the modulation of the GM1 associated activity affects an allergic condition and/or a hypersensitivity condition.

3. The use of an agent according to claim 1 or claim 2 wherein the agent is capable of blocking an IgE mediated response.

4. An assay method for identifying an agent according to any one of claims 1 to 3 that is capable of affecting an allergic condition and/or a hypersensitivity condition;

wherein the assay method comprises:

(a) contacting an agent with a ganglioside receptor;

(b) determining whether the agent modulates a ganglioside associated activity;

such that the modulation of the ganglioside associated activity is indicative that the agent may be capable of affecting an allergic condition and/or a hypersensitivity condition; and

wherein the agent is not coupled to an antigen.

5. An assay method according to claim 4 wherein the assay is to screen for an agent useful in the prevention and/or treatment of an allergic condition and/or a hypersensitivity condition.

6. A process comprising the steps of:

(a) performing the assay according to claim 4 or claim 5;

(b) identifying one or more agents capable of modulating a ganglioside associated activity; and

(c) preparing a quantity of those one or more agents.

7. A process comprising the steps of:

(a) performing the assay according to claim 4 or claim 5;

(b) identifying one or more agents capable of modulating a ganglioside associated activity; and

(c) preparing a pharmaceutical composition comprising those one or more identified agents.

8. A process comprising the steps of:

(a) performing the assay according to claim 4 or claim 5;

(b) identifying one or more agents capable of modulating a ganglioside associated activity; and

(c) modifying one or more identified agents that modulates a ganglioside associated activity; and

(d) preparing a pharmaceutical composition comprising those one or more modified agents.

9. An agent identified by the process of claim 6 or claim 7 or claim 8.

10. An agent according to claim 9 wherein the agent is a GM1 binding agent.

11. An agent according to claim 10 wherein the agent is EtxB.

12. An agent according to claim 9 wherein the agent had not previously been known to affect an allergic condition and/or a hypersensitivity condition through modulation of a ganglioside associated activity.

13. A ganglioside according to claims 4 to 8 wherein the ganglioside is a GM1 ganglioside.

14. A method of affecting an allergic condition or a hypersensitivity condition with one or more agents;

wherein the agent is capable of modulating a ganglioside associated activity in an *in vitro* assay method; and

wherein the *in vitro* assay method is the assay method defined in claim 4 or claim 5.

15. Use of an agent according to any one of claims 4 to 14 in the manufacture of a medicament to affect an allergic condition and/or a hypersensitivity condition.

16. An agent according to claim 9 or claim 10 or claim 11 or claim 12 prepared by a process according to claim 6 or claim 7 or claim 8 for use as a pharmaceutical.

17. A pharmaceutical composition comprising or prepared from an agent according to any one of claims 9 or claim 10 or claim 11 or claim 12 or an agent prepared by a process according to claim 6 or claim 7 or claim 8.

18. Use of an agent in the preparation of a pharmaceutical composition according to claim 7 or claim 8 or claim 16 or claim 17 for the treatment of an allergic condition and/or a hypersensitivity condition.

19. An agent capable of modulating a ganglioside associated activity substantially as described herein.

DECLARATION AND POWER OF ATTORNEY

CTH-03

As below-named inventors, we hereby declare that:

Our residences, post office addresses, and citizenships are as stated below next to our names.

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled **Agent for Treating Allergic or Hypersensitivity Condition**, the specification of which is attached hereto.

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. To the best of our knowledge, information, and belief the facts stated therein are true.

We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

We hereby claim foreign priority benefits under Title 35, United States Code §119 of the following foreign applications:

GB 9800487.2, filed 9 January 1998 and
PCT/GB99/00070, filed 8 January 1999.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(1)

We hereby appoint Mary M. Krinsky, Registration No. 32,423, 79 Trumbull Street, New Haven, CT 06511-3708 (203-773-9544), with full power of substitution, association and revocation, as attorney to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Please direct all telephone calls and correspondence to Mary M. Krinsky at the above address and telephone number.

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